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EXAMINER	
CANELLA, KAREN A	

ART UNIT	PAPER NUMBER
1642	29

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.	Applicant(s)
08/973,303	Dormer
Examiner	**Art Unit**
Karen Canella	1642

-- *The MAILING DATE of this communication appears on the cover sheet with the correspondence address* --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3 months* MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☐ Responsive to communication(s) filed on _____.

2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.

3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under ***Ex parte Quayle***, 1935 C.D. 11; 453 O.G. 213.

Disposition of Claims

4) ☒ Claim(s) *36-39, 41, 42, 45-67, and 69-72* is/are pending in the application.

4a) Of the above, claim(s) *48-61 and 63-65* is/are withdrawn from consideration.

5) ☐ Claim(s) _____ is/are allowed.

6) ☒ Claim(s) *36-39, 41, 42, 45-47, 62, 66, 67, and 69-72* is/are rejected.

7) ☐ Claim(s) _____ is/are objected to.

8) ☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9) ☒ The specification is objected to by the Examiner.

10) ☐ The drawing(s) filed on _____ is/are a) ☐ accepted or b) ☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12) ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13) ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a) ☐ All b) ☐ Some* c) ☐ None of:

1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

*See the attached detailed Office action for a list of the certified copies not received.

14) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

a) ☐ The translation of the foreign language provisional application has been received.

15) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s). _____
4) ☒ Interview Summary (PTO-413) Paper No(s). *29*
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other

DETAILED ACTION

1. After review and reconsideration, the finality of the Office action of Paper No. 23 is withdrawn.

2. Claims 36-38, 42, 42, 62 and 72 have been amended. Claims 36-39, 41, 42, 45-67 and 69-72 are pending. Claims 48-61 and 63-65 remain withdrawn from consideration. Claims 36-39, 41, 42, 45-47, 62 and 66, 67 and 69-72 are under consideration.

3. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

4. The disclosure is objected to because of the following informalities: the pages of the disclosure are not numbered. Section § 1.52 of the M.P. E. P. States

> Other than in a reissue application or reexamination proceeding, the pages of the specification including claims and abstract must be numbered consecutively, starting with 1, the numbers being centrally located above or preferably, below, the text..

Appropriate correction is required.

5. The specification is objected to as not complying with 1.821(d) of the Sequence Rules and Regulations. When the specification of a patent application discusses a sequence listing that is set forth in the "Sequence Listing" in accordance with paragraph (c) of the Sequence Rules and Regulations, reference must be made to the sequence by use of the assigned identifier, in the text of the description or claims of the patent application. The specification recites clones by name such as DY-8 and 2.2 kb eda, HA-15/2 and HA-12/5. DY-8 is identified only once in Figure 20 as SEQ ID NO:2; 2.2 kb eda is identified only once in the Brief Description of Figure 18 as SEQ ID NO:1. The specification makes no reference to SEQ ID NO:4. Appropriate correction is required.

6. Claims 36-39, 41, 42, 45-47, 62 and 66, 67 and 69-72 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 36 specifies a protein comprising the properties recited in sections a through e. It is unclear if these properties are referred to in the alternative, or if these properties are referred to as collective properties of the claimed protein. For purpose of examination, both options will be considered. It is noted that page one of the specification describes the differentiation-inducing activity as having at least the features of a, b, c and d, wherein feature a is isolation from murine myelomonocytic cell lines; feature b, is isolation from irradiated human bone marrow cell lines; feature c is the induction of differentiation of Friend Leukemia cells with hemoglobin formation and feature d is a molecular weight in the range of 10 kDa to 60 kDa as determined by gel filtration. It would appear that the features associated with claim 36, such as c, d and e were not presented by the specification as required features of the claimed activity.

Claim 36 recites "An isolated protein with differentiation-inducing activity on Friend Erythroleukemia cell lines with hemoglobin formation" and as such does not specify where the hemoglobin is formed. Claim 36, part d further recites "is encoded by a cDNA comprising repeat sequences of SEQ ID NO:6 and 7". It is unclear if the cDNA is to contain multiple copies of SEQ ID NO:6 and 7, or if SEQ ID NO:6 and 7 comprise said "repeat sequences". For purpose of examination, both alternatives will be considered. Further it is unclear if multiple species of mRNA comprise the same coding region of SEQ ID NO:2, or if a single species within the multiple species encompassed by part e encodes a single protein.

Claim 36 is vague and indefinite in the recitation of "corresponding mRNA". It is unclear if "corresponding" refers to the mRNA encoding the protein, or if corresponding refers to homologous mRNA from other species. For purpose of examination, both alternatives will be considered.

Claim 37 is objected to for failing to further limit the scope of claim 36. Claim 37 recites properties which are inherent in the protein of claim 36.

Claim 37 is vague and indefinite in the recitation of "protein...which shows an in vitro up regulation and/or accumulation if a three day allogenic spleen cell reaction is carried out". By stating "if a three day reaction is carried out" rather than "when a three day reaction is carried out" the metes and bounds of the claim is unclear, as any protein, including the instant eda protein, will not show up regulation or accumulation in spleen cells without the allogenic reaction. Further, claim 37 is vague and indefinite as the up regulation and/or accumulation is not linked to a specific cell or organ. Claim 37 is indefinite in the recitation of part b, "having AT rich regions in the cDNA, the 3' part of which encodes the protein". Firstly "AT rich regions" is a relative term not defined by the specification, secondly, it is unclear if applicant intends to claim AT rich proteins which are in the 3' part of the protein or specifically extraneous to said 3' part.

Claim 38 is rendered vague and indefinite in the recitation of "repeat sequences hybridizing to SEQ ID NO:6, 7, 8, 9 or 10". It is unclear if the sequences hybridizing to SEQ ID NO:6, 7, 8, 9 or 10 must be sequences which are multiple copy in order to satisfy the requirement of "repeat sequence". Further, limitations for hybridization conditions which do not specify the physical parameters of the washing steps are not limiting as many polynucleotides will hybridize to the DNA encoding the protein, but will be eliminated under conditions of washing. Thus, the metes and bounds of claim 38 cannot be determined.

Claim 39 is vague and indefinite in the recitation of human cells or human cell lines. Claim 39 embodies the protein of claim 36 which specifies that the corresponding mRNA comprises the coding region of SEQ ID NO:2. SEQ ID NO:2 is taught by the specification to be a murine protein originating from WEHI-B cells. It is unclear how claim 39 can depend on claim 36.

Claim 41 is objected to for the typographical error of "cDNA or SEQ ID NO:1" rather than "cDNA of SEQ ID NO:1". For purpose of examination the claim will be read as "cDNA of SEQ ID NO:1"

Claim 41 is objected to for failing to further limit the scope of claim 36. Claim 41 is drawn to a protein comprising a partial amino acid sequence encoded by a DNA hybridizing to a fragment of SEQ ID NO:1, 2 or 4. Claim 36 specifies that the corresponding mRNA for the

claimed protein comprises the coding region of SEQ ID NO:2. Claim 41 is drawn to fragments of SEQ ID NO:2 which are smaller than the claimed coding region, and therefore encompasses a genus of proteins which is broader in scope than claim 36.

Claim 41 is vague and indefinite in the recitation of "stringent conditions" . Without limitations as to the chemical and physical conditions to be employed for the hybridization, the term "stringent" does not serve to limit the metes and bounds of the claim. Claim 41 is also rendered vague and indefinite in the recitation of "said differentiation inducing activity" with regard to claim 36. It is unclear if "said differentiation inducing activity" refers to all of the limitations of claim 36 in parts a through e, or if it refers only to part a. For purpose of examination, all alternatives will be considered.

Claim 42 is objected to for failing to further limit the scope of claim 36. Claim 42 is drawn to variants of the protein of claim 36 and thus is drawn to a genus of proteins which is non-overlapping with the genus of proteins encompassed by claim 36. Furthermore, claim 42 specifies "differentiation inducing activity on Friend Leukemia cell line" which fulfills only the specific embodiment of claim 36 part a.

Claim 45 is objected to for failing to further limit the scope of claim 36 by specifying growth factor activity and colony stimulating activity. These are inherent properties of the protein of claim 36 having corresponding mRNA comprising the coding region of SEQ ID NO:2 as disclosed by the specification.

Claim 46 is objected to for failing to further limit the scope of claim 36. Claim 46 specifies that said protein has a differentiation-inducing activity on human leukemia cell lines which is an inherent property of the protein of claim 36 having corresponding mRNA comprising the coding region of SEQ ID NO:2 as disclosed by the specification.

Claim 47 is vague and indefinite in the recitation of "partial amino acids according to SEQ ID NO:3 or SEQ ID NO:5". It is unclear if the partial amino acid sequence is a fragment of SEQ ID NO:3 or 5, or if the partial amino acid sequence comprises SEQ ID NO:3 or SEQ ID NO:5. For purpose of examination, both alternatives will be considered.

Claim 47 is objected to for failing to further limit the scope of claim 36. Claim 46 is drawn to a protein comprising fragments of SEQ ID NO:3 and 5. Claim 36 specifies that the protein has a mRNA corresponding to the coding region of SEQ ID NO:2 SEQ ID NO:2 encodes SEQ ID NO:3, therefore only a protein comprising the full coding region of SEQ ID NO:2 would have the specific limitations of claim 36. Further, claim 47 appears to be drawn to variants of SEQ ID NO:3 and 5, wherein each variant having differentiation inducing activity on Friend Leukemia cell lines. Claim 36 specifies that the differentiation inducing activity had the specific limitation of sections a through e. A protein having only the limitation of part a, as in claim 47, would not further limit claim 36.

The metes and bonds of claim 62 cannot be determine because it is unclear if the "differentiation-inducing activity" of section a refers only to the induction of differentiation on Friend Leukemia cell lines, or if it refers to all the limitations in sections a through e of claim 36.

Claim 66 is vague and indefinite in the recitation of "human or murine protein with differentiation inducing activity on Friend erythroleukemia cell lines according to claim 36". It is unclear if all the limitations of claim 36, sections a through e are encompassed in claim 66 as "differentiation inducing activity" or if only section a is encompassed in the instant claim. Further, it is unclear how a human protein meets the limitations of claim 36 drawn to a corresponding mRNA having the coding region of SEQ ID NO:2, as said coding region is from a murine protein.

The recitation of stringent hybridization conditions in claim 67 without specific limitations of physical and chemical parameters of said hybridization does not limit the claimed protein. It is unclear if the differentiation inducing activity of claim 67 comprises all the limitations of claim 36 from sections a through e, or if the differentiation inducing activity on Friend leukemia cell lines refers only to part a of claim 36. Further, it is unclear if claim 37 comprises the protein of claim 36, and further comprises "an amino acid sequence at least a part of which is encoded by a DNA sequence hybridizing to the DNA sequence according to SEQ ID NO:1, 2, or 4", as an additional amino acid sequence or if the limitations of claim 36 drawn to "an amino acid sequence at least a part of which is encoded by a DNA sequence hybridizing to the DNA sequence according to SEQ

ID NO:1, 2, or 4" is a characterization of the protein of claim 36. For purpose of examination, both alternatives will be considered.

Claim 67 is objected to for failing to further limit claim 36. Claim 36 includes the limitation that the corresponding mRNA comprises the coding region of SEQ ID NO:2. An alignment of SEQ ID NO:1, 2 and 4 indicates that nucleotides 329-674 of SEQ ID NO:2 are the same as nucleotides 277 to 622 of SEQ ID NO:4 and nucleotides 1110 to 1455 of SEQ ID NO:1. Thus, it appear that claim 36 already embodies an amino acid sequence at least a part of which is encoded by a DNA hybridizing to SEQ ID NO:1, 2 or 4, and thus claim 67 will not further limit claim 36.

Claim 69 is objected to for failing to limit claim 36 as growth factor activity, colony-stimulating activity, the induction of erythropoiesis and the immune system are inherent properties of the claimed protein as taught by the specification.

Claim 69 recites "inducing the immune system" as a property of the protein of claim 36, however, the outcome or object of said induction is not stated.

Claim 69 is objected to for errors in syntax. A growth factor, a colony stimulating factor and a factor inducing erythropoiesis are products not properties.

Claim 70 is objected to for failing to further limit claim 36. Claim 36 embodies the limitation that the corresponding mRNA comprises the coding region of SEQ ID NO:2. The specification teaches that the coding region of SEQ ID NO:2 is nucleotides 155 to 686. Claim 70 is drawn in part to a protein comprising amino acids which are encoded only by nucleotides 74 to 154 of SEQ ID NO:2 as nucleotides 74-154 are referred to in the alternative, and would be outside of the scope of claim 36.

Claim 71 is vague and indefinite in the recitation of "repeat sequences" as it is unclear if "repeat sequences" refers to multiple copies of SEQ ID NO: 6-10 or if "repeat sequences refers to repeated nucleotides within SEQ ID NO:6-10.

Claim 71 is objected to for failing to further limit the scope of claim 36. Claim 46 is drawn to a protein wherein the corresponding mRNA comprises the coding region of SEQ ID

NO:2. Claim 71 specifies that the repeat sequences of SEQ ID NO:6-10 or sequences hybridizing thereto are present within the DNA encoding the protein of claim 36. However, an alignment of SEQ ID NO:6-10 with SEQ ID NO:2 in its entirety indicate that one copy of SEQ ID NO:6, 7 and 10 is present within SEQ ID NO:2. Further, the the alignment revealed that SEQ ID NO:8 has no similarity between SEQ ID NO:1, 2 or 4 and SEQ ID NO:9 has no similarity between SEQ ID NO:2 or 4. It is unclear how SEQ ID NO:9 and 8 can be present within the DNA encoding the protein of claim 36 . Thus, it is reasonable to conclude that the scope of claim 71 is neither co-extensive nor limiting to the scope of claim 36.

Claim 72 is vague and indefinite in the recitation of "corresponding mRNA". It is unclear if "corresponding" refers to the mRNA encoding the protein, or if corresponding refers to homologous mRNA from other species. For purpose of examination, both alternatives will be considered. In addition, claim 72 specifies a protein comprising the properties recited in sections a through e. It is unclear if these properties are referred to in the alternative, or if these properties are referred to as collective, inherent properties of the claimed protein. For purpose of examination, both options will be considered. Claim 72 recites "An isolated protein with differentiation-inducing activity on Friend Erythroleukemia cell lines with hemoglobin formation" and as such does not specify where the hemoglobin is formed. Claim 72, part d further recites "is encoded by a cDNA comprising repeat sequences of SEQ ID NO:6 and 7". It is unclear if the cDNA is to contain multiple copies of SEQ ID NO:6 and 7, or if SEQ ID NO:6 and 7 comprise said "repeat sequences". For purpose of examination, both alternatives will be considered. Further it is unclear if multiple species of mRNA comprise the same coding region of SEQ ID NO:2, or if a single species within the multiple species encompassed by part e encodes a single protein. Additionally, the limitation of washing the filter at 60 degrees in an aqueous solution having a salt concentration of 15mM NaCl and a concentration of SDS of 0.1% is not limiting without recitation of the length of the wash step.

7. Claims 36-39, 41, 42, 45-47, 62 and 66, 67 and 69-72 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for proteins and fusion proteins comprising SEQ ID NO:2 or SEQ ID NO:4, does not reasonably provide enablement for other proteins which can be cloned from the eda activity characterized in that the differentiation inducing activity is dependent on a serum factor in fetal calf serum and characterized by an increase in mRNA species having variable 5' regions and 3' regions corresponding to the coding regions of SEQ ID NO:2 or therapeutic compositions comrpising SEQ ID NO:1, 2, or 4, or variants or fragments of SEQ ID NO:1, 2, or 4. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The first paragraph of 35 U.S.C. 112 states that "the specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same...". The courts have interpreted this to mean that the specification must enable one skilled in the art to make and use the invention without undue experimentation. The courts have further interpreted undue experimentation as requiring "ingenuity beyond that to be expected of one of ordinary skill in the art" (Fields v. Conover, 170 USPQ 276 (CCPA 1971)) or requiring an extended period of experimentation in the absence of sufficient direction or guidance (In re Colianni, 195 USPQ (CCPA 1977)). Additionally the courts have determined that "...where a statement is , on its face, contrary to generally accepted scientific principles", a rejection for failure to teach how to make/or use is proper (In re Marzocchi, 169 USPQ 367 (CCPA 1971)). Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 U.S.C. 112, first paragraph have been described in In re Colianni, 195 USPQ 150, 153 (CCPA 1977) and have been clarified by the Board of Patent Appeals and Interferences in Ex parte Forman, 230 USPQ 546 (BPAI 1986). Among the factor are the nature of the invention, the state of the prior art, the predictability or lack thereof in the art, the amount of direction or guidance present, the presence or absence of

working examples, the breadth of the claims, and the quantity of experimentation needed. The instant disclosure fails to meet the enablement requirement for the following reasons:

The instant invention is drawn to an isolated protein with differentiation-inducing activity on Friend Leukemia cell lines. The specification has identified two clones SEQ ID NO:2 and SEQ ID NO:4 that comprise opening reading frames and potentiate the differentiation of Friend Leukemia cells. The specification describes the use of SEQ ID NO:2 in the isolation of a larger cDNA fragment called HA-15/2, which after transfection into COS cells exhibited a weak differentiation-inducing effect. The specification teaches that "the gene" is expressed in the form of different mRNA species which are presumed to be splice variants (page 2). The specification has identified another clone of SEQ ID NO:1 which, although longer than SEQ ID NO:2 or 4, does not contain an open reading frame, but exerts a weak differentiation inducing activity when transfected in vivo. The specification discloses that a differentiation-inducing activity is composed of a proteins of from 10 kDa to 60 kDa, corresponding to mRNA of various sizes and inducible in the WEHI-3B mouse cell line when the cell line is exposed to an undefined serum factor which is present in variable amounts in fetal calf serum. The specification terms the collection of proteins having differentiation inducing activity as "eda" (erythroid differentiation inducing activity). The specification states that "An identical activity with respect to its effect has also been discovered in the supernatant of irradiated human bone marrow cells". The specification gives no details on the isolation of the claimed activity from human bone marrow cells, thus it is assumed that it also requires the same unidentified serum factor as the aforesaid mouse cell line. The specification does not identify this serum factor and states that said serum factor is present in variable amount in fetal calf serum. It is reasonable to conclude that some batches of fetal calf serum will be either devoid of the serum factor, or have a low level of serum factor that fails to activate the production of the claimed differentiation inducing activity. Thus, without an identification of the serum factor that causes the production of the claimed differentiation inducing activity, one of skill in the art would be subject to undue experimentation without reasonable expectation of success, in order to isolate other proteins comprised by the activity beyond those

encoded by SEQ ID NO:2 and 4 as the serum factor necessary for the production of the activity may be absent from the serum used to culture the cells. Further, for the reasons set forth in the rejection of claim 36 under 112, second paragraph above, the claim is drawn in part to an isolated polypeptide which encoded by SEQ ID NO:6 and 7. Said Sequence identifiers represent polynucleotide of 42 and 55 base pairs, which would encode a twelve amino acid peptide and a twenty-eight amino acid peptide. These peptides are present in SEQ ID NO:1, 2 and 4, which are 1495, 715 and 636 nucleotides in length, respectively. The specification states on page 3 that because the differentiation inducing effect on erythropoietic cells was discovered using clone DO-8 which contains about 640 bp of the 3' region of eda together with 73 bp of the specific 5' end the differentiation inducing activity is associated with the 3' end of the cDNA. The brief description of figure 20 identifies DY-8 as SEQ ID NO:2. There is no objective evidence in the specification that the peptides encoded by SEQ ID NO:6 and 7, which are fragments of SEQ ID NO:2 and 4, can function as a differentiation-induing activity independently of the complete amino acid sequences of SEQ ID NO:2 and 4, or that other proteins which comprise polynucleotides containing SEQ ID NO:6 and 7 would also have differentiation inducing activity. The specification does not provide any evidence that the peptides encoded by nucleotides 467 to 509 and 523 to 577 of SEQ ID NO:2 would retain the differentiation inducing activity on Friend Leukemia cells, alone, or in the context of a heterologous amino acid sequence. It is well known in the art that proteins are folded 3-dimensional structures, the function and stability of which are directly related to a specific conformation (Mathews and Van Holde, Biochemistry, 1996, pp. 165-171). In any given protein, amino acids distant from one another in the primary sequence may be closely located in the folded, 3-dimensional structure (Mathews and Van Holde, Biochemistry, 1996, pp. 166, figure 6.1). The specific conformation of a protein results from non-covalent interactions between amino acids, beyond what is dictated by the primary amino acid sequence. A different amino acid sequence surrounding the fragments encoded by SEQ ID NO:6 and 7 can potentially radically alter the three dimensional structural environment in which the given fragment is located (Matthews, B. Genetic and Structural Analysis of the Protein Stability Problem, In:

Perspectives in Biochemistry, Hans Neurath, Ed., 1989, Vol. 1, pp. 6-9) thus, the consequences of the altered sequence environment cannot be predicted. Additionally, the proteins encoded by SEQ ID NO:2 and 4 are secreted into the cell culture medium, but the instant claims are not limited to secreted proteins. It is recognized in the art that protein function is context dependent, and cellular aspects, such as membrane anchorage, protein activation and sub-cellular location must be considered with respect to protein function in addition to molecular aspects (Bork, Genome research, 2000, Vol. 10, pp. 398-400, especially page. 398, under the heading "Limitations in the Total Knowledge Base of Protein Function"). Thus, it is reasonable to conclude that possession of SEQ ID NO:6 and 7, or the other claimed fragments of SEQ ID NO:8-10, would not guarantee that a membrane bound protein would exert that same differentiation inducing activity as the proteins encoded by SEQ ID NO:2 and 4.

Claims 42 and 62 are specifically drawn to the variants of claim 36. However, the specification lacks teachings of how to make said variants which encode proteins which retain the differentiation-inducing activity of SEQ ID NO:3 and 5. Neither the specification nor the claims limits the number of amino acid substitutions, deletions and additions which are encompassed in the claimed variant protein. Thus, the number of structural changes which are encompassed by the claims is unlimited. As stated above, proteins are folded 3-dimensional structures, the function and stability of which are directly related to a specific conformation (Mathews and Van Holde, Biochemistry, 1996, pp. 165-171). In any given protein, amino acids distant from one another in the primary sequence may be closely located in the folded, 3-dimensional structure (Mathews and Van Holde, Biochemistry, 1996, pp. 166, figure 6.1). The specific conformation of a protein results from non-covalent interactions between amino acids, beyond what is dictated by the primary amino acid sequence. Thus, the resulting consequence of any given amino acid change is dependent upon what is substituted for the original amino acid and the three dimensional structural environment in which the given amino acid is located (Matthews, B. Genetic and Structural Analysis of the Protein Stability Problem, In: Perspectives in Biochemistry, Hans Neurath, Ed., 1989, Vol. 1, pp. 6-9). Often, when altering the amino acid sequence of a

protein, a second alteration is necessary to restore the function of the protein. For example in hemoglobin, a mutation of Asp to Asn at position beta 99 results in a abnormal hemoglobin. In normal hemoglobin the Asp at position beta 99 is stabilized by a Try at position alpha 42 and an Asn at position alpha 97. The normal function of the mutated hemoglobin can be restored by producing a double mutant retaining the first mutation of Asn at position 99 beta in addition to substituting a Asp for Tyr at position alpha 42 (Kim et al, PNAS, 1994, Vol. 91, pp. 11547-11551). As another example of the interactions of amino acids in a 3-dimensional protein structure, Frish et al (Biol. Chem., Hoppe-Seyler, 1994, 375:353-356) observed that a human Vk protein of an antibody is destabilized after a substitution of Cys 23 . This de-stabilization was found to be reversed by a substitution of Try for His at position 32. Frish concluded that there was a stabilizing interaction (non-covalent interaction) between the Cys 23 and the Tyr 32 in the original antibody. To further illustrate the unpredictability of the art with regard to alteration of protein sequences, it is noted that replacement of a single lysine reside at position 118 of acidic fibroblast growth factor by glutamic acid led to the substantial loss of heparin binding, receptor binding and biological activity of the protein (Burgess et al. Journal of Cell Biology, 1990, Vol. 111, pp.2129-2138, cited in a previous Office action). Considering transforming growth factor alpha, it is noted that replacement of aspartic acid at position 47 with alanine or asparagine did not affect biological activity while replacement with serine or glutamic acid sharply reduced the biological activity of the mitogen (Lazar et al. Molecular and Cellular Biology, 1998, Vol. 8, pp.1247-1252, cited in a previous Office action). These references demonstrate that even a single amino acid substitution amounting to a small chemical modification will often dramatically affect the biological activity and characteristic of a protein. Reasonable correlation must exist between the scope of the claims and scope of enablement set forth, and it cannot be predicted from the disclosure how to make variant polypeptides from variant polynucleotides which have the differentiation-inducing activity of SEQ ID NO:2.

Therefore, as the presence of an adequate amount of the unidentified serum factor cannot be guaranteed in a batch of serum, and as one of skill in the art could not anticipate the functional

activity of a protein which minimally comprises a peptide encoded by SEQ ID NO:6-10, variants of SEQ ID NO:1, 2, 4, or variants of the fragments of SEQ ID NO:6-10, one of skill in the art would be forced into undue experimentation in make and use the broadly claimed proteins.

Claim 62 is drawn to a therapeutic composition comprising the protein of claim 36, or variant or fragment of said protein, wherein said variant or fragment retains said differentiation inducing activity and a conventional carrier and/or excipient in an amount effective to treat diseases accompanied by impairment of differentiation inducing activity in erythropoietic cells. The claim lacks enablement for variants and fragments for the reasons set forth above. Further, the claim also lacks enablement for a therapeutic composition comprising the proteins encoded by SEQ ID NO:1, 2 and 4. The specification has provided no evidence that administration of composition comprising SEQ ID 3 or 5 in vivo, to a patient suffering from erythroleukemia would have a therapeutic effect. It appears that proteins of SEQ ID NO:3 and 5 are not selective for only cancerous erythrocytes nor would it be expected that the proteins would act only on cancerous erythrocytes to the exclusion of normal erythrocyte pre-cursors. Thus, administration of the claimed therapeutic composition could result in the differentiation of all erythrocyte precursors, both normal and cancerous, and the impact of this non-selectivity on a patient can not be predicted. Further, it is expected that a therapeutic agent administered in vivo for the treatment of a leukemia must be administered to attain a sufficient concentration for a sufficient length of time. Variables such as biological stability, half-life or clearance from the blood are important parameters in achieving successful therapy. The composition may be inactivated in vivo before producing a therapeutic effect, for example, by protease degradation, immunological de-activation or due to an inherently short half life of the administered proteins It is clear, that the specification does not address these issues or teach a therapeutic dose for SEQ ID NO:3 and 5 for the treatment of erythroleukemia ...The specification provides insufficient guidance with regard to these issues and no evidence has been provided which would allow one of skill in the art to make and use the claimed therapeutic compositions in vivo with a reasonable expectation of success.

8. Claims 36-38, 39, 41, 42, 47, 67, 69 and 72 are rejected under 35 U.S.C. 102(b) as being anticipated by Eto et al (Biochemical and Biophysical Research Communications, 1987, Vol. 142, pp. 1095-1103, reference of the IDS submitted 28 November 1999) as evidenced by the abstract of Horton et al (Blood, 1962, Vol. 20, pp. 302-313) and Accession numbers NM_002192 and NP_002183. Claim 36 is drawn to an isolated protein with differentiation-inducing activity on Friend Leukemia cells. Claim 37 is drawn in part to the protein of claim 37 wherein said protein has AT rich regions in the cDNA, the 3' part of which encodes the protein. Claim 38 embodies the protein of claim 36 wherein repeat sequences which hybridize to SEQ ID NO:6, 7, 8, 9 or 10 under stringent conditions are present in the DNA encoding the protein. Claim 39 specifies that the protein according to claim 36 is isolated for human cells, murine cells or the culture supernatant of human cells or murine cells. Claim 41 is drawn to the protein of claim 36 wherein said protein comprises a partial amino acid sequence encoded by a DNA hybridizing to a fragment of the cDNA of SEQ ID NO:1, 2, or 4, wherein said fragment retains aid differentiation-inducing activity. Claim 42 is drawn in part to a variant of claim 36, wherein said variant retains said differentiation inducing activity. Claim 47 embodies the protein of claim 36 wherein said protein has partial amino aid sequence according to SEQ ID NO:3 or 5, wherein one or more of the amino acid sequence may be deleted, substituted or added each having at least differentiation inducing activity on Friend erythroleukemia cell lines. Claim 67 is drawn to the protein of claim 36 comprising an amino acid sequence at least a part of which is encoded by the DNA sequence hybridizing to the DNA sequence according to SEQ ID NO:1 or 2 or 4 under stringent conditions. Claim 69 embodies the protein of claim 36, wherein said protein comprises a growth factor, a colony-stimulating factor or a factor inducing erythropoiesis. Claim 71 embodies the protein of claim 36 wherein one or more of repeat sequences hybridizing to SEQ ID NO:6 or 7 under stringent conditions are present in the DNA encoding the protein. Claim 72 is drawn in part to an isolated protein with differentiation-inducing activity on Friend Leukemia cells comprising the property of induction of differentiation in Friend Leukemia cells with hemoglobin formation and a molecular weight in the range of about 10 kDa to 60 k Da.

Eto et al disclose erythroid differentiation factor of Mr 15 kDa which induced the differentiation of Friend Leukemia cells (page 1101, lines 13-14). The Mr weight of 15 kDa is within the claimed range. Eto et al measured the differentiation by means of staining with dianisine which is known to be an indicator of hemoglobin as evidenced by the abstract of Horton et al. It is noted for the reasons stated in the rejection of claim 36 under 112, second paragraph, that it is unclear if the properties of sections a through e of claims 36 and 72 are referred to in the alternative or if all the properties in sections a through e are required in the claimed protein and that for purpose of examination the claim will be read as encompassing the limitations of sections a through e in the alternative. With this interpretation of the claim language, it is clear that Eto et al disclose a protein with differentiation inducing activity on Friend Leukemia cells having a molecular weight in the range of 10 kDa to 60 kDa, thus fulfilling the specific embodiments of claims 36 and 72.

With regard to claim 37, it is noted that the metes and bounds of of the claim cannot be determined in reference to the exact constitution of an AT rich region for the reasons set forth in the rejection under 112, second paragraph, above. Accession number NP_002183 discloses that the coding sequence for EDF is nucleotides 18-1366 of Accession Number NM_002192. Inspection of the nucleotide sequence of the 3' region fof NM_002192 following the coding sequence shows that the cDNA possesses an AT rich region from residue 1421 to 1840. Therefore, it is reasonable to conclude that the EDF of Eto et al fulfills the specific embodiments of claim 37 with respect to part b, as the protein has AT rich regions in the cDNA. Further, as the 3' part can be considered to start in the middle of the cDNA and run to the 3' end, the 3' part of the EDF sequence encodes the carboxyl terminus of the EDF protein, therefore all the limitations of claim 37, section b have been met.

Eto et al also fulfills the specific embodiments of claims 38 and 71 with regard to repeat sequences which hybridize to SEQ ID NO: 6, 7, 8, 9, 10 under stringent conditions which are present in the DNA encoding the protein, as the repeat sequence of "aaaaaaa" (residues 1491-1497) would hybridize under stringent conditions to nucleotides 164-170 (ttttttt) of SEQ ID

NO:9 and the repeat sequences of residues 371-374 "gggg" would hybridize under stringent conditions to resides 24-27 (cccc) of SEQ ID NO:6. Eto et al fulfills the specific embodiments of claim 39 with regard to the isolation of the protein from human cells (abstract).

Eto et al disclose the specific embodiment of claim 41. EDF comprises a partial amino acid sequence which is encoded by a DNA hybridizing to a fragment of the cDNA of SEQ ID NO:1, wherein said fragment retains said differentiation inducing activity. EDF comprises a partial amino acid sequence encoded by nucleotides 371-374 (gggg) which is a DNA hybridizing to a fragment of SEQ ID NO:1 (cccc, residues 1208-1211) wherein said fragment retain differentiation-inducing activity. It is inherent that the 3' end of SEQ ID NO:1 has differentiation inducing activity.

Eto et al disclose the specific embodiments of claim 42 with respect to variants of claim 36 as neither the claim nor the specification limit the number of substitutions, deletions or additions that can be made to produce a variant protein. The amino acid sequence of EDF can thus be considered a variant of the protein encoded by SEQ ID NO:2 as EDF retains differentiation inducing activity of Friend Leukemia cells.

Eto et al disclose the limitations of claim 47 with respect to a protein comprising partial amino acid sequences according to SEQ ID NO:3 or 5, wherein one or more amino acids may be deleted substituted or added each having differentiation inducing activity on Friend Leukemia cell lines as the EDF of Eto et al comprises the amino acid sequence of "RR" at residues 306-307 which is a partial amino acid sequence in SEQ ID NO:3 and 5 (residues 102-103 and 136-137, respectively).

Eto et al fulfills the specific embodiments of claim 67, as EDF comprises an amino acid sequence "RR" at residues 306-307 which is apart of said amino acid sequences which would be encoded by DNA sequence hybridizing to SEQ ID NO:2 or 4 under stringent conditions because residues 1004-1008 of EDF encode "RR", said DNA encoded to RR would hybridize to the DNA sequence of residues 458-463 of SEQ ID NO:2 and residues 306-311 of SEQ ID NO:4 .

Eto et al disclose the specific embodiment of claim 69 with respect to a factor inducing erythropoiesis as EDF causes differentiation of erythroleukemia cell lines measured by hemoglobin formation, and is thus an inducer of erythropoiesis (page 1096, last paragraph, under the heading "Bioassay for EDF").

9. All other rejections and objections as set forth in Paper No. 23 are withdrawn.

10. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Karen Canella whose telephone number is (703) 308-8362. The examiner can normally be reached on Monday through Friday from 8:30 am to 6:00 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anthony Caputa, can be reached on (703) 308-3995. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

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